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REVIEW ARTICLE

Brain Dialysis (Microdialysis)

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Abstract

Brain dialysis or microdialysis is a minimally-invasive sampling technique that is used for continuous measurement of free, unbound analyte concentrations in the extracellular fluid of virtually any tissue. Analytes may include endogenous molecules (e.g. neurotransmitter, hormones, glucose, etc.) to assess their biochemical functions in the body, or exogenous compounds (e.g. pharmaceuticals) to determine their distribution within the body. The microdialysis technique requires the insertion of a small microdialysis catheter (also referred to as microdialysis probe) into the tissue of interest. The microdialysis probe is designed to mimic a blood capillary and consists of a shaft with a semipermeable hollow fiber membrane at its tip, which is connected to inlet and outlet tubing. The probe is continuously perfused with an aqueous solution (perfusate) that closely resembles the (ionic) composition of the surrounding tissue fluid at a low flow rate of approximately 0.1- 5ìL/min. Once inserted into the tissue or (body) fluid of interest, small solutes can cross the semipermeable membrane by passive diffusion. The direction of the analyte flow is determined by the respective concentration gradient and allows the usage of microdialysis probes as sampling as well as delivery tools. The solution leaving the probe (dialysate) is collected at certain time intervals for analysis.

Keywords: Brain Dialysis; Microdialysis; Microdialysis Catheter; Microdialysis Probe; Dialysate; Neurotransmitter; Semipermeable Membrane.

Introduction

Brain dialysis, also called microdialysis, is a new technique based on the push-pull cannula. It can be used for continuously perfusing and collecting perfusate of certain brain areas in freely moving animals. Coupled with high performance liquid chromatography (HPLC) and radioimmunoassay (RIA), brain dialysis is allowed to determine the extracellular changes of many neurotransmitters in the brain, such as acetylcholine, noradrenaline, dopamine, 5-HT and their metabolites, free amino acids, small peptides, phosphoethanolamine, vitamins, various ions and so on.

Need of Microdialysis

Micro dialysis is a minimally-invasive sampling technique that is used for continuous measurement of free, unbound analyte concentrations in the extracellular fluid of virtually any tissue. Analytes may include endogenous molecules (e.g. neurotransmitter, hormones, glucose, etc.), to assess their biochemical functions in the body, or exogenous compounds (e.g. pharmaceuticals) to determine their distribution within the body. The microdialysis technique requires the insertion of a

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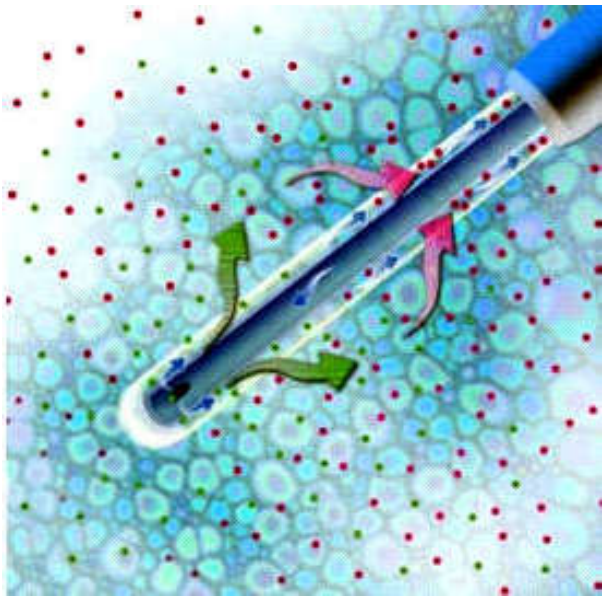


Fig. 1:

History of Brain Dialysis

The microdialysis principle was first employed in the early 1960s, when push-pull canulas and dialysis sacs were implanted into animal tissues, especially into rodent brains, to directly study the tissues' biochemistry [1]. While these techniques had a number of experimental drawbacks, such as the number of samples per animal or no/limited time resolution, the invention of continuously perfused dialytrodes in 1972 helped to overcome some of these limitations. Further improvement of the dialytrode concept resulted in the invention of the "hollow fiber", a tubular semipermeable membrane with a diameter of ~200-300μm, in 1974. Today's most prevalent shape, the needle probe, consists of a shaft with a hollow fiber at its tip and can be inserted by means of a guide cannula into the brain and other tissues.

Principles of Microdialysis

- Microdialysis is a minimally invasive technique to explore and monitor the chemistry in blood and living tissues.
- Continuous tissue monitoring is enabled through the insertion of small Microdialysis probes. A physiological salt solution is slowly constantly pumped through a semipermeable membrane and the solution is equilibrated with the surrounding tissue fluid.
- The Microdialysis Catheter mimics a blood capillary. Substances from the extracellular fluid of the tissue diffuse across the membrane of the catheter into the perfusion fluid inside the catheter.
- The main design resembles a concentric tube where the perfusion fluid enters through an inner tube; flows to its distal end; exits the tube and enters the space between the inner tube and the outer dialysis membrane. This is where the "dialysis" takes place, i.e. the diffusion of molecules between the extra cellular fluid and the perfusion fluid.
- Microdialysis offers the opportunity to sample tissue chemistry with high accuracy and without taking any blood. The dialysate is extracted into small vials for bedside or laboratory analysis.

Fig. 2:

Features and Procedures of Brain in Microdialysis

The micro dialysis catheter takes up substances delivered by the blood e.g. glucose and drugs, but also substances released from the cells e.g. markers of cellular metabolism. Substances may also be introduced into the tissue by including them in the perfusate, e.g. precursors of enzymatic processes, and the products of the process may then be recovered by the microdialysis catheter.

Another unique possibility is to deliver drugs to the tissue for studies of pharmacodynamic effects or for obtaining local therapeutic effect. Treatment of brain tumors have been attempted using this approach.

The high recovery of substances that can be achieved in the human brain makes it possible to analyze most neurotransmitters and energy metabolites but also cytokines (Hillman et al., 2005) and small proteins using catheters with a cut off of 100 kDa as compared to the conventional catheter with 20 kDa cut off. The two catheters give equivalent results for small molecular substances such as glucose, lactate, pyruvate, and glutamate and lactate/ pyruvate ratio. The high cut off microdialysis catheters can, therefore, be used for routine clinical monitoring of extracellular substances (Hutchinson et al., 2005), as well as for research on e.g. larger molecular weight peptides.

Techniques of Brain Dialysis

The technique of micro dialysis enables sampling and collecting of small-molecular-weight substances from the interstitial space. It is a widely used method in neuroscience and is one of the few techniques available that permits quantification of neurotransmitters, peptides, and hormones in the behaving animal. More recently, it has been used in tissue preparations for quantification of neurotransmitter release.

This unit provides a brief review of the history of microdialysis and its general application in the neurosciences. The authors review the theoretical principles underlying the microdialysis process, methods available for estimating extracellular concentration from dialysis samples (i.e., relative recovery), the various factors that affect the estimate of in vivo relative recovery, and the importance of determining in vivo relative recovery to data interpretation.

Several areas of special note, including impact of tissue trauma on the interpretation of microdialysis results, are discussed. Step-by-step instructions for the planning and execution of conventional and quantitative microdialysis experiments are provided. Microdialysis is a technique for continuous sampling of the interstitial fluid chemistry of tissues and organs. It is minimally

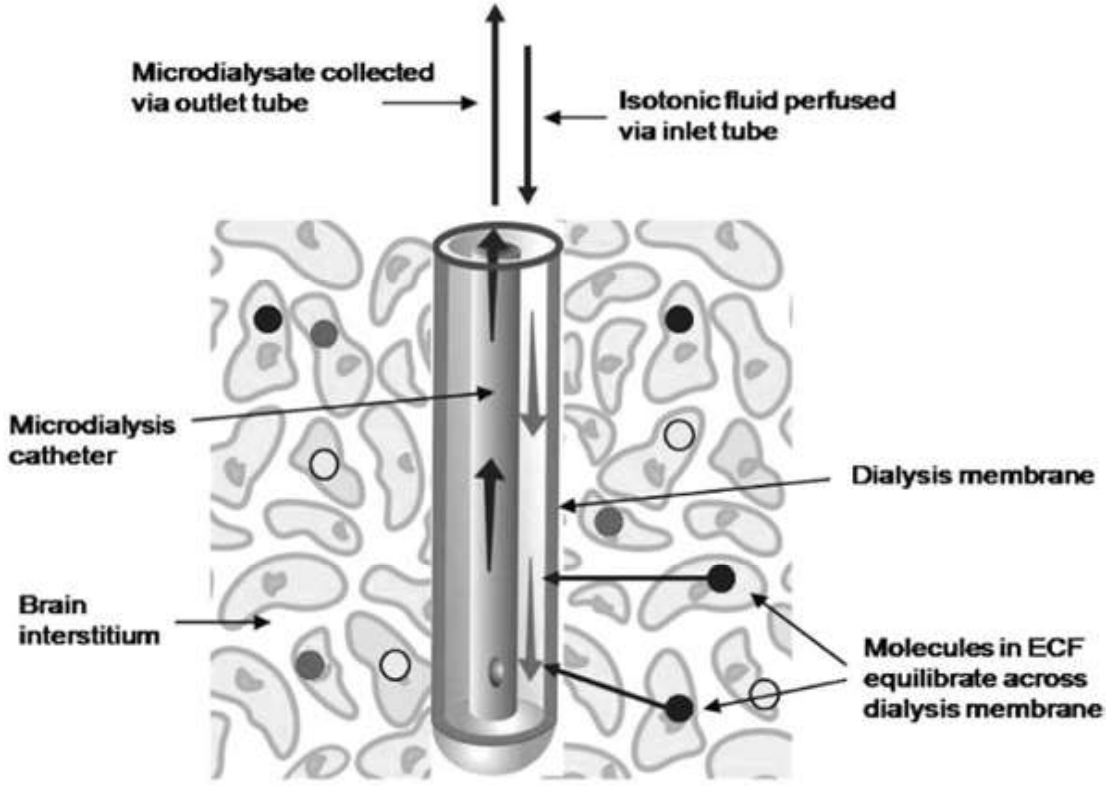


Fig. 3:

invasive and simple to perform in a clinical setting. The microdialysis catheter samples all small molecular substances present in the interstitial fluid, however, the use of microdialysis in neurointensive care has focused on markers of ischemia and cell damage.

Lactate/Pyruvate ratio (LPR) is a marker of changes in the redox state of cells and a ratio >25 is an early warning of ischemia and mitochondrial dysfunction. Glycerol is a marker of cell membrane decomposition.

Loss of energy due to ischemia and/or mitochondrial dysfunction eventually leads to an influx of calcium and a decomposition of cell membranes, which liberates glycerol into the interstitial fluid.

Brain glucose is an important marker due to the increasing interest in controlling blood glucose within defined limits. Low systemic glucose may cause brain hypoglycemia during neuro-intensive care leading to secondary brain damage.

Glutamate may open neuronal calcium channels initiating a pathological influx of calcium thus provoking cell damage. However, most likely an increase in extracellular glutamate signals an energy deficiency causing a decreased uptake of glutamate into astrocytes.

The Penumbra, i.e. tissue adjacent to a focal lesion, is considerably more vulnerable than normal brain tissue. A microdialysis catheter positioned in the penumbra detects early signs of ischemia and mitochondrial damage that may lead to cell damage. After SAH a microdialysis catheter in the tissue at risk will detects vasospasm hours before clinical signs are evident. Microdialysis predicts outcome in SAH, TBI and MCA patients and is used increasingly as a tool to individualize patient treatment in routine neurointensive care.

Recovery and Calibration Methods

Low-Flow-Rate Method

The low-flow-rate method is based on the fact that the extraction efficiency is dependent on the flow-rate. At high flow-rates, the amount of drug diffusing from the sampling site into the dialysate per unit time is smaller (low extraction efficiency) than at lower flow-rates (high extraction efficiency). At a flow-rate of zero, a total equilibrium between these two sites is established (Cout = Csample). This concept is applied for the (low-)flow-rate method, where the probe is perfused with blank perfusate at different flow-rates. Concentration at the sampling site can be determined by plotting the extraction ratios against the corresponding flow-rates and extrapolating to zero-flow. The low-flow-rate method is limited by the fact that calibration times may be rather long before a sufficient sample volume has been collected.

No-net-Flux-Method

During calibration with the no-net-flux-method, the microdialysis probe is perfused with at least four different concentrations of the analyte of interest (Cin) and steady-state concentrations of the analyte leaving the probe are measured in the dialysate (Cout). The recovery for this method can be determined by plotting Cout in over Cin and computing the slope of the regression line. If analyte concentrations in the perfusate are equal to concentrations at the sampling site, no-net flux occurs. Respective concentrations at the no-net-flux point are represented by the x-intercept of the regression line. The strength of this method is that, at steady-state, no assumptions about the behaviour of the compound in the vicinity of the probe have to be made, since equilibrium exists at a specific time and place. However, under transient conditions (e.g. after drug challenge), the probe recovery may be altered resulting in biased estimates of the concentrations at the sampling site. To overcome this limitation, several approaches have been developed that are also applicable under non-steady-state conditions. One of these approaches is the dynamic no-net-flux method.

Dynamic No-Net-Flux Method

While a single subject/animal is perfused with multiple concentrations during the no-net-flux method, multiple subjects are perfused with a single concentration during the dynamic no-net-flux (DNNF) method. Data from the different subjects/animals is then combined at each time point for regression analysis allowing determination of the recovery over time. The design of the DNNF calibration method has proven very useful for studies that evaluate the response of endogenous compounds, such as neurotransmitters, to drug challenge.

Retro Dialysis

During retrodialysis, the microdialysis probe is perfused with an analyte-containing solution and the disappearance of drug from the probe is monitored. The recovery for this method can be computed as the ratio of drug lost during passage

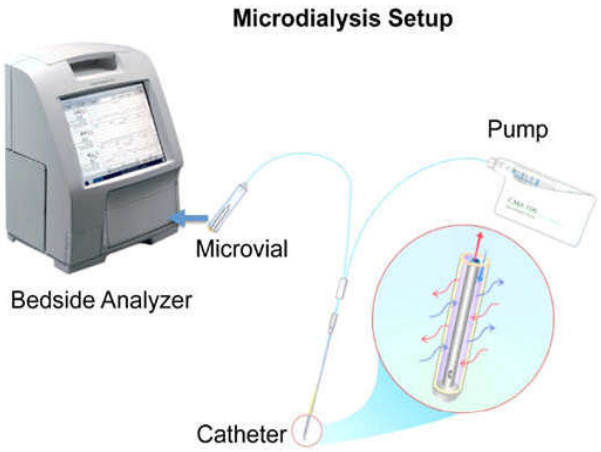
(Cin Cout) and drug entering the microdialysis probe (Cin). In principle, retrodialysis can be performed using either the analyte itself (retrodialysis by drug) or a reference compound (retrodialysis by calibrator) that closely resembles both the physiochemical and the biological properties of the analyte. Despite the fact that retrodialysis by drug cannot be used for endogenous compounds as it requires absence of analyte from the sampling site, this calibration method is most commonly used for exogenous compounds in clinical settings.

Applications

- To monitor concentrations of endogenous biomolecules in the brain
- Micro dialysis is commonly used to measure neurotransmitters their metabolites, as well as small neuromodulators and energy substrates
- To monitoring free concentrations of endogenous as well as exogenous compounds in virtually any tissue
- It is increasingly employed in humans to monitor free, unbound drug tissue concentrations as well as interstitial concentrations of regulatory cytokines and metabolites
- Applications in other organs include the skin and monitoring of glucose concentrations in patients with diabetes

Advantages

1. To date, microdialysis is the only sampling technique that can continuously monitor drug or metabolite concentrations in the extracellular fluid of virtually any tissue. Depending on the exact application, analyte concentrations can be monitored over several hours, days, or even weeks. Free, unbound extracellular tissue concentrations are in many cases of particular interest as they resemble pharmacologically active concentrations at or close to the site of action. Combination of microdialysis with modern imaging techniques, such positron emission tomography, further allow for determination of intracellular concentrations.
2. Insertion of the probe in a precise location of the selected tissue further allows for evaluation of extracellular concentration gradients due to transporter activity or other factors, such as perfusion differences. It has, therefore, been suggested as the most appropriate technique to be used for tissue distribution studies
3. Exchange of analyte across the semipermeable membrane and constant replacement of the sampling fluid with fresh perfusate prevents drainage of fluid from the sampling site, which allows sampling without fluid loss. Microdialysis can consequently be used without disturbing the tissue conditions by local fluid loss or pressure artifacts, which can occur when using other techniques, such as microinjection or push-pull perfusion.
4. The semipermeable membrane prevents cells, cellular debris, and proteins from entering into the dialysate. Due to the lack of protein in the dialysate, a sample clean-up prior to analysis is not needed and enzymatic degradation is not a concern.



Limitations

1. Despite scientific advances in making microdialysis probes smaller and more efficient, the invasive nature of this technique still poses some practical and ethical limitations. For example, it has been shown that implantation of a microdialysis probe can alter tissue morphology resulting in disturbed microcirculation, rate of metabolism or integrity of physiological barriers, such as the blood-brain barrier While acute reactions to probe insertion, such as implantation traumas, require sufficient recovery time, additional factors, such as necrosis, inflammatory responses, or wound healing processes have to be taken into consideration for long-term sampling as they may influence the experimental outcome. From a practical perspective, it has been suggested to perform microdialysis experiments within an optimal time window, usually 24–48 hours after probe insertion.
2. Microdialysis has a relatively low temporal and spatial resolution compared to, for example, electrochemical biosensors. While the temporal resolution is determined by the length of the

sampling intervals (usually a few minutes), the spatial resolution is determined by the dimensions of the probe. The probe size can vary between different areas of application and covers a range of a few millimeters (intracerebral application) up to a few centimeters (subcutaneous application) in length and a few hundred micrometers in diameter.

3. Application of the microdialysis technique is often limited by the determination of the probe's recovery, especially for in vivo experiments. Determination of the recovery may be time-consuming and may require additional subjects or pilot experiments. The recovery is largely dependent on the flow rate: the lower the flow rate, the higher the recovery. However, in practice the flow rate cannot be decreased too much since either the sample volume obtained for analysis will be insufficient or the temporal resolution of the experiment will be lost. It is therefore important to optimize the relationship between flow rate and the sensitivity of the analytical assay. The situation may be more complex for lipophilic compounds as they can stick to the tubing or other probe components, resulting in a low or no analyte recovery.

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